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Association between rs1799724 of TNF- α gene and early onset preeclampsia in Chinese: A pilot study

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ABSTRACT

Objective: To investigate the association between polymorphisms of TNF- α (rs1799724, rs1800629), VEGF (rs3025039) and VEGFR1 (rs 722503) and early onset preeclampsia (EOPE) in Chinese.
Methods: A total of 132 EOPE patients from January 2016 to December 2018 at the Second Hospital of Tianjin Medical University were selected as the EOPE group, and 156 normal pregnant patients as the Control group. In both groups, 5 ml of peripheral venous blood was obtained after admission. The characteristics of genotype and allele distribution at the four SNPs in the study subjects were examined by matrix-assisted laser desorption ionization time-of-flight mass spectrometric genotyping.
Results: The genotype frequency distribution and allele frequency distribution of rs1799724 were significantly different between the EOPE group and the Control group ($P = 0.002$, $P = 0.003$). The T allele was statistically associated with the development of EOPE under a dominant genetic model ($P = 0.001$). The genotype and allele frequency distributions of rs1800629, rs3025039, and rs 722503 did not differ significantly between the EOPE group and the Control group ($P > 0.05$). There was no linkage disequilibrium among rs1799724, rs1800629 and rs3025039 loci, the corresponding haploid cannot be formed.
Conclusions: The rs1799724 of TNF- α gene is a genetic susceptibility locus for EOPE and may be a potential predictors of preeclampsia.

1. Introduction

Preeclampsia (PE) is a hypertensive disorder of pregnancy-specific syndrome, and affects approximately 2%– 5% pregnant women [1,2]. PE can severely affect maternal and child health and is a major cause of increased maternal morbidity and mortality [3]. Early-onset preeclampsia (EOPE) refers to preeclampsia with onset before 34 weeks of gestation. Early-onset, rapid progression, severe disease, and poor prognosis are the characteristics of EOPE. Because EOPE occurs early and severely compromises maternal and child health, it is now the focus of research today. The pathogenesis of EOPE is extremely complex and has not been elucidated, and clinical symptoms, once present, have caused irreparable organ damage to the mother and child, seriously compromising the health of the mother and child. Currently, there is currently no effective treatment for EOPE, while the mainstay remains symptomatic management. Therefore, the exploration of methods for the early prediction of EOPE has become a research hotspot.

Genetic factors play a crucial role in the pathogenesis of EOPE. Research on the genetic etiology of EOPE has made some progress. A large number of single nucleotide polymorphisms (SNPs) in PE susceptibility genes have been reported successively in studies conducted in different countries and populations. But the results varied greatly between different studies, which may be related to the different backgrounds of genetic factors in different ethnic populations [4]. However, most studies suggest that multiple SNPs collectively contribute to the onset and progression of EOPE and then determine the genetic susceptibility to EOPE and the variability of drug response. The study of SNP loci has important implications for the pathogenesis as well as for early prediction of EOPE. Early risk prediction can reduce morbidity, mortality, and improve clinical outcomes in patients with EOPE [5]. Tumor

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necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF) have been identified to be associated with placental vascular endothelial injury, hemodynamic changes, and disturbed angiogenesis, and are important contributing factors to the development of PE.

In this study, genetic polymorphisms of VEGF (rs3025039), VEGFR1 (rs 722503), and TNF- α (rs1799724, rs1800629) on chromosome 6 were investigated for their association with the development of severe early-onset preeclampsia. The study of gene polymorphisms and genetic immunological relationships may lead us to understand the pathogenesis of EOPE at the genetic level and provide a new basis for early prediction and gene therapy of EOPE.

2. Subjects and methods

2.1. Study subjects

A total of 132 women with EOPE who were hospitalized and delivered in the Department of Obstetrics, Tianjin Medical University Second Hospital between January 2018 and December 2020 were included in this study. Written informed consent was obtained from each subject before blood samples were taken.

The investigation protocol conformed to the Declaration of Helsinki and was approved by the ethics committee of The Second Hospital of Tianjin Medical University.

Data from 132 subjects with were collected in the EOPE group. The patient's diagnosis was performed according to the ISSHP classification, diagnosis and management recommendations for international practice [6]. The detailed diagnostic criteria were: gestational hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg) after 20 weeks of gestation, urinary protein ≥ 300 mg / 24 h, or urinary protein (+) accompanied by upper abdominal discomfort, headache, blurred vision, or other maternal organ dysfunction. All subjects in the control group without hypertension [6].

Demographic data (including age, blood pressure, gestational weeks, and body mass index), levels of albumin, and 24-h urine protein were collected. Five milliliters of peripheral venous blood from all patients were collected and placed in EDTA anticoagulant tubes and then frozen at − 80 ℃ for DNA extraction. The details of the subjects in both groups are shown in Table 1.

2.2. SNP selection and inclusion criteria

SNP inclusion criteria: (1) the SNP locus has demonstrated significant association with PE in at least 2 or more independent studies; (2) SNP loci with a minimum allele frequency greater than or equal to 1% in the Chinese population; (3) The SNP loci studied are located in promoter and exonic regions and have an impact on gene function. DbSNP database were used in this study (http://www.ncbi.nlm.nih.gov/snp). Ultimately, finally four SNPs (rs3025039, RS 722503, rs1799724, rs1800629) were included.

Table 1
Comparison of baseline data between the two groups.

Variables	Control group	EOPE group	*P*
n	156	132	
Age (years)	30.89 ± 5.38	30.21 ± 4.93	0.263
Gestational weeks (wk)	32.24 ± 2.68	39.21 ± 0.97	< 0.001
Body mass index (kg/m^2)	32.09 ± 2.95	28.04 ± 3.89	< 0.001
Systolic blood pressure (mmHg)	163.18 ± 19.60	114.58 ± 7.12	< 0.001
Diastolic blood pressure (mmHg)	111.75 ± 15.17	76.02 ± 5.30	< 0.001
Albumin (g/L)	29.29 ± 3.29	37.48 ± 2.71	< 0.001
24-h urine protein (g/24 h)	10.30 ± 6.75	0.00 ± 0.00	< 0.001

2.3. Genotyping by matrix-assisted laser desorption ionization time of flight mass spectrometric

2.3.1. Primer design and synthesis

The gene sequence of VEGF (rs3025039), VEGFR1 (rs 722503) and TNF-α(rs1799724, rs1800629) were obtained through the my Agena website (https://memory-beta.fandom.com/wiki/Agena). The Assay-Designer 3. 1 software was used to design primers. PCR reaction primer synthesis were performed by Beijing Liuhe BGI Technology Co., Ltd. and shown in Table 2.

2.3.2. Genotyping

Genomic DNA from whole blood was extracted using Magnetic Bead System (BioTeke Corporation Co.,Beijing, China) following the manufacturer's instructions.

All the SNPs were obtained by the improved multiple ligase detection reaction method. TYPER genotyping software 4. 0 was used to obtain the relevant data and genotyping plots, after which data were exported and saved.

2.4. Statistical methods

Plink analysis software (http://www.cog-genomics.org/plink/1.9/) was performed for the SNP association analysis. The Hardy-Weinberg equilibration was detected for the Control group. Chi-square (χ^2) tests were applied to detect differences in the distribution of genotype frequencies and allele genotype frequencies between the Control group and the EOPE group. Logistic regression analyzes were performed with dominant models and logistic regression analyses with recessive models. Linkage disequilibrium and haplotype analyzes were performed using Hapview analysis software (http://sourceforge.net/projects/haploview/). The linkage disequilibrium between loci was examined by D'/r^2.

3. Results

3.1. Hardy-Weinberg equilibrium testing in the control group

First, we analyzed whether the genotype frequency distribution of each SNP locus in the control group was consistent with the Hardy-Weinberg equilibrium. The frequency of VEGF (rs3025039), VEGFR1 (rs 722503) and TNF- α (rs1799724, rs1800629) was in Hardy-Weinberg equilibration ($P > 0.05$). The results are detailed in Table 3.

3.2. TNF- α (rs1799724) genotype and allele in the two groups

The TNF- α (rs1799724) genotype frequencies of C/C, C/T, and T/T were 68.2%, 31.1%, and 0.7% in the EOPE group cases and 84.6%, 14.1%, and 1.3% in the Control group, respectively, and the genotype frequency distribution between the two group was statistically different ($P = 0.002$). The C allele frequency was 83.7% and the T allele frequency was 16.3% in the EOPE group. In the Control group, the frequency of the C allele was 91.7%, the frequency of the T allele was 8.3%, and the frequency distribution of the T allele was significantly different between the two groups ($P = 0.003$, OR=2.140).

The association between the T allele of TNF- α (rs1799724) and EOPE was further analyzed under the dominant inheritance model. The results showed that there was a statistical association between T allele and EOPE under the dominant inheritance model ($P = 0.001$, OR=2.567). However, these results were not found in recessive genetic models ($P = 0.662$, OR=0.588). (Table 4).

The genotyping map of the TNF- α (rs1799724) in the blood of the subjects are shown in Supplementary Figure 1.

3.3. TNF- α (rs1800629) genotype and allele in the two groups

There were no statistical differences in the TNF-α (rs1800629)

Table 2
Primers applied in the analysis of SNPs in the study.

SNP_ID	2nd-PCRP	1st-PCRP	UEP_SEQ
rs1799724	ACGTTGGATGCTATGGAAGTCGAGTATGGG	ACGTTGGATGGTATTCCATACCTGGAGGTC	GGGACCCCCCCTTAA
rs1800629	ACGTTGGATGAAGGAAACAGACCACAGACC	ACGTTGGATGGATTTGTGTGTAGGACCCTG	GCGGTTTTGAGGGGCATG
rs3025039	ACGTTGGATGATGGCGAATCCAATTCCAAG	ACGTTGGATGACTCTGCGCAGAGCACTTTG	TAATTCCAAGAGGGACC
rs 722503	ACGTTGGATGAGATTGTCCATGGCAAGGAG	ACGTTGGATGATCCACCCTTTCCATTCCAC	TTCAGACAGGACTGGAGGGAAGG

Table 3
Hardy-Weinberg equilibration in the control group.

Gene	SN	Genotype	χ^2	*P*
TNF-α	rs1799724	T/C	0.923	0.336
TNF-α	rs1800629	A/G	1.251	0.263
VEGF	rs3025039	T/G	0.181	0.671
VEGFR1	rs722503	C/T	4.126	0.042

Table 4
Comparison of TNF-α rs1799724 genotype and allele between the two groups.

	Control group	EOPE group	χ^2	*P*	OR (95%CI)
n	156	132			
Genotype					
C/C	132(84.6)	90(68.2)	12.093	0.002	1(Reference)
C/T	22(14.1)	41(31.1)			2.733(1.526-4.897)
T/T	2(1.3)	1(0.7)			0.733(0.066-8.209)
Allele					
T	26(8.3)	43(16.3)	8.581	0.003	2.140(1.276-3.591)
C	286(91.7)	221 (83.7)			
Dominant model					
C/T + T/T	24(15.4)	42(31.8)	10.931	0.001	2.567(1.454-4.532)
C/C	132(84.6)	90(68.2)			
Recessive model					
T/T	2(1.3)	1(0.8)	0.191	0.662	0.588(0.053-6.556)
C/C+C/T	154(98.7)	131 (99.2)			

genotype frequencies of G/G, A/G, and A/A in the EOPE group and the distribution in the control group, respectively ($P = 0.993$). The frequency of the G allele was 95.1%, the A allele was 4.9% in the EOPE group, the frequency of the G allele was 95.2%, and the A allele was 4.8% in the control group, both of which were not statistically different ($P = 0.948$, OR = 0.975).

We further explored and analyzed the association A allele of TNF-α (rs1800629) with EOPE pathogenesis under a dominant genetic model and a recessive genetic model, respectively. And the results indicated that the A allele was not associated with EOPE (all $P > 0.05$) (see Table 5 for details). The genotyping of TNF-α (rs1800629) in the blood of the subjects are shown in Supplementary Figure 2.

3.4. VEGF(rs3025039) genotype and allele in the two groups

There was no statistical difference in the VEGF (rs3025039) genotype frequencies of C/C, C/T and T/T in the EOPE group and the distribution in the control group, respectively ($P = 0.404$). The frequency of the C allele was 79.8%, the T allele was 20.2% in the EOPE group, the frequency of the C and T allele was 81.7% and 18.3% in the control group, both of which were not statistically different ($P = 0.555$, OR = 1.135).

The association of T allele of VEGF (rs3025039) with EOPE pathogenesis under a dominant genetic model and a recessive genetic model was analyzed respectively. And the results showed that the T allele was not associated with EOPE (all $P > 0.05$) (Table 6). The genotyping of VEGF (rs3025039) in the blood of the subjects are shown in Supplementary Figure 3.

Table 5
Comparison of TNF-α (rs1800629) genotype and allele between the two groups.

Variables	Control group	EOPE group	χ^2	*P*	OR (95%CI)
n	156	132			
Genotype					
G/G	142(91.0)	120(90.9)	0.014	0.993	1(Reference)
A/G	13(8.3)	11(8.3)			0.999(0.432-2.311)
A/A	1(0.6)	1(0.8)			0.84(0.052-13.655)
Allele					
G	297(95.2)	251(95.1)	0.004	0.948	0.975(0.455-2.088)
A	15(4.8)	13(4.9)			
Dominant model					
G/G	142(91.0)	120(90.9)	0.001	0.973	0.986(0.439-2.213)
A/G+A/A	14(9.0)	12(9.1)			
Recessive model					
G/G+A/G	155(99.4)	131(99.2)	0.014	0.906	0.845(0.52-13.644)
A/A	1(0.6)	1(0.8)			

3.5. VEGFR1 (rs 722503) genotype and allele in the two groups

There was no statistical difference in the VEGFR1(rs722503)

Table 6
Comparison of the VEGF (rs3025039) genotype and allele between the two groups.

Variables	Control group	EOPE group	χ^2	*P*	OR (95%CI)
n	156	132			
Genotype					
C/C	105(67.3)	78(61.9)	1.813	0.404	1(Reference)
C/T	45(28.8)	45(35.7)			1.346(0.811-2.234)
T/T	6(3.8)	3(2.4)			0.673(0.163-2.775)
Allele					
T	57(18.3)	51(20.2)	0.349	0.555	1.135(0.745-1.729)
C	255(81.7)	201(79.8)			
Dominant model					
C/T + T/T	51(32.7)	48(38.1)	0.893	0.345	1.267(0.775-2.070)
C/C	105(67.3)	78(61.9)			
Recessive model					
T/T	6(3.8)	3(2.4)	0.484	0.486	0.610(0.149-2.488)
C/C+C/T	150(96.2)	123(97.6)			

genotype frequencies of T/T, C/T and C/C in the EOPE group and the control group ($P = 0.149$). There were no statistical differences between the frequency of the T and A allele in the EOPE group and in the control group ($P = 0.042$, OR = 0.677). There was no statistical association between the VEGFR1(rs722503) T allele and EOPE either in a dominant genetic model or recessive genetic model (all $P > 0.05$) (Table 7).

The genotyping of VEGFR1(rs722503) in the blood of the subjects are shown in Supplementary Figure 3.

3.6. Linkage disequilibrium analysis

The r^2 value of the three loci on chromosome 6 (rs1799724, rs1800629, rs3025039) is 0, and there is no linkage disequilibrium. Therefore, the corresponding haploid cannot be formed, so the haploid analysis cannot be performed (Fig. 1).

4. Discussion

Since the known TNF-α, VEGF and VEGFR play a decisive role in EOPE pathogenesis [7] [8] [9]. Therefore, this study analyzed the relationship of the above three genetic SNPs with EOPE. We found that rs l799724 was associated with an increased risk of EOPE. This study contributes to the elucidation of the genetic basis of the pathogenesis of EOPE. At the same time, it is also able to provide a theoretical basis for early recognition, early intervention, and reduction of the complication rate of EOPE, and even gene therapy for EOPE.

The TNF- α gene is located on chromosome 6. The promoter gene polymorphisms of TNF- α have a close relationship with the occurrence and development of preeclampsia [10,11]. TNF- α Gene polymorphisms may have an effect on cytokine expression. Increased plasma TNF- α expression levels can cause placental trophoblast apoptosis and release a large number of coagulation factors, which contribute to the pathogenesis of preeclampsia [12]. In this study, we investigated the SNPs at rs1800629 and rs1799724. Among them, the genotype distribution at rs1800629 was not statistically different between the EOPE group and the control group.

However, Madhavi Puppala et al. [13] study on PE and control subjects in northern India showed that the distribution of the rs18000629 GG genotype was significantly different between PE case ($n = 100$) and control subjects($n = 100$) ($P = 0.005$), which was inconsistent with the conclusion of our study. On the one hand, it may be related to ethnic differences, or it may be related to the relatively small sample size included in their research. Meanwhile, the studies of Mohammadpour -Gharehbagh A [14], Zubor P [15] and Saarela T [16] were consistent with the conclusions of this study. None of them fund significant differences in the allele frequencies of rs1800629 between PE and control subjects.

Table 7
Comparison of VEGFR1(rs722503) genotype and allele between the two groups.

Variables	Control group	EOPE group	χ^2	P	OR (95%CI)
n	156	132			
Genotype					
T/T	99(63.5)	69(52.3)	3.814	0.149	1(Reference)
C/T	45(28.8)	48(36.3)			0.653(0.392-1.088)
C/C	12(7.7)	15(11.4)			0.558(0.246-1.265)
Allele					
T	243(77.9)	186(70.5)	4.153	0.042	0.677(0.465-0.986)
C	69(22.1)	78(29.5)			
Dominant model					
T/T	99(63.5)	69(52.3)	3.683	0.055	0.631(0.393-1.011)
C/T + C/C	57(36.5)	63(47.7)			
Recessive model					
T/T + C/T	144(92.3)	117(88.6)	1.134	0.287	0.650(0.293-1.443)
C/C	12(7.7)	15(11.4)			

More importantly, we found that the rs1799724 locus genotype distribution was statistically different ($P = 0.002$). There was a statistically significant difference in the distribution of the T allele ($P = 0.003$), indicating that the T allele significantly increased the risk of EOPE. Under the dominant genetic model, CT / TT genotypes had a significantly increased risk of developing EOPE compared to CC ($P = 0.001$, OR= 2.567). Thus, it is seen that the T allele is the causative factor for EOPE.

The pathogenesis of PE may be related to angiogenic pathways. The gene of VEGF is also located on chromosome 6, and VEGF has a strong promoting effect on endothelial cell growth [17,18]. VEGF single nucleotide polymorphism changes can cause angiogenesis disturbance, which in turn leads to the occurrence of PE [19,20]. VEGFR1 and VEGFR2, the main receptors responding to VEGF, are mainly distributed in the vascular endothelium and can stimulate endothelial cell growth and improve vascular permeability [21]. It has been confirmed that familial genetic variations of VEGFR may play a role in the PE pathogenesis. In this study, the loci rs3025039 in VEGFA gene and rs722503 in VEGFA (FLT1) gene were screened for study. We found that there were no statistical differences between the genotypes and the T allele of rs3025039 and rs722503 in EOPE and control subjects. Therefore, it showed that the loci rs3025039 and rs722503 were not associated with genetic susceptibility to EOPE in the pregnant women we included.

But there is a contradiction in the current study on the relationship of rs3025039 and rs722503 with EOPE. As in the study of Melissa D. Amosco et al. [22] on Filipino pregnant women, the difference of allele frequency distribution of rs3025039 between PE group ($n = 150$) and normal control ($n = 175$) is not statistically significant ($P = 0.0626$), inferring that the SNP rs3025039 is not associated with PE in Filipino pregnant women. Similarly, in an Iran study by Amin-Beidokhti M et al. [23] also found that the rs3025039 and allele genotypes were not significantly different. While, in the study of Sudanese women by Hamid HM et al. [24], the rs3025039 SNP was significantly associated with PE onset ($P = 0.01$), where the T allele was significantly higher in EOPE group ($P = 0.02$). In 2016, Amosco M [25] et al. also showed that the rs3025039C allele was associated with PE incidence (OR = 1.648) and the C allele increased the risk of PE in Filipino pregnant women.

Similarly, mutually antagonistic conclusions emerge from studies on rs722503. Melissa D. Amosco et al. [22] study in Filipino pregnant women, the allele frequency distribution of rs722503 in the PE group ($n = 150$) and the normal pregnant group ($n = 175$) was not statistically significant. In contrast, Srinivas SK et al.[26], analyzed data from 489 black women and 117 white females data suggested that the rs722503 polymorphism is associated with PE incidence in them. It follows that differences in human ethnicity may be key to the inconsistent conclusions drawn from the current studies regarding rs3025039 and rs722503.

For the results of linkage disequilibrium analysis, only loci with $D' > 0.8$ and $r^2 > 0.8$ were in accordance with the strong linkage genetic relationship, haplotypes could be constructed and analyzed for haplotype frequency difference. The r^2 values of three loci on chromosome 6 (rs1799724, rs1800629, rs3025039) in this study were 0, which did not have a strong linkage genetic relationship and therefore could not compose the corresponding haplotypes.

In conclusion, rsl799724 was associated with the risk of EOPE. Our finding may be of some help for early prediction and intervention of EOPE, and more studies are expected to further confirm this conclusion.

Ethics approval and consent to participate

The study was obtained permission from the Ethics Committee of the

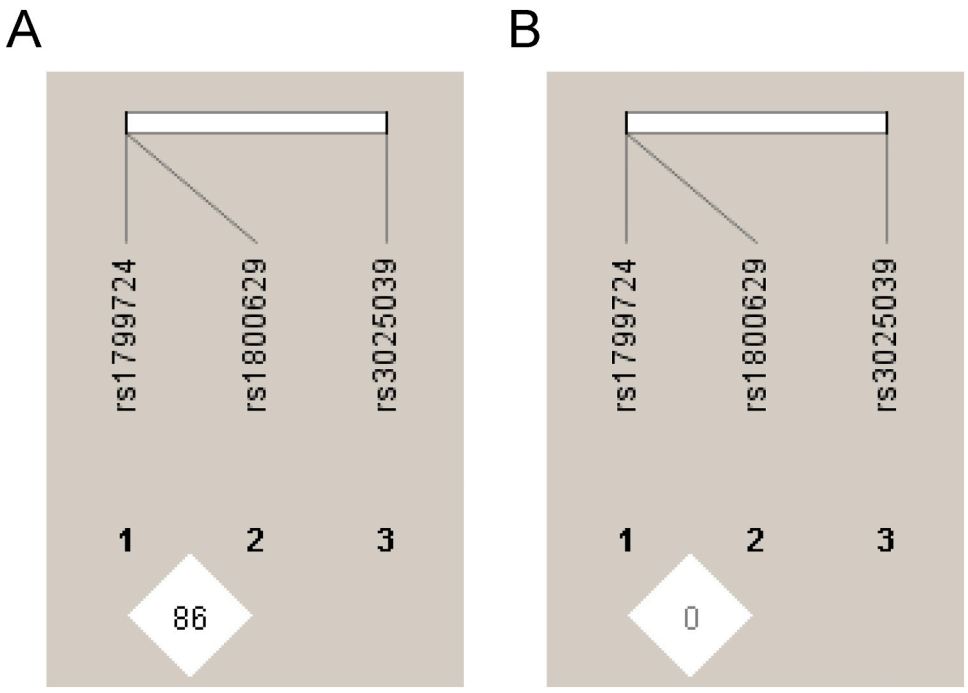

Fig. 1. Linkage disequilibrium map of three SNP loci on chromosome 6. A, D′= 0.86; B, $r^2 = 0.000$.

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CRediT authorship contribution statement

Yujie Wang: Writing – original draft, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Jianheng Bao:** Writing – original draft, Software, Formal analysis, Data curation, Conceptualization. **Shaofang Hua:** Investigation, Data curation. **Lirong Yin:** Writing – review & editing, Methodology, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

Not applicable.

Data access statement

All data generated and used are presented in this article.

Consent for publication

Not applicable.

Authors contributions

All authors contributed significantly to this study. Yujie Wang and Lirong Yin designed the trial. Yujie Wang, Shaofang Hua and Lirong Yin have conducted the work and are involved in data collection. Yujie Wang and Jianheng Bao analyzed and interpreted the data. Yujie Wang and Jianheng Bao wrote the manuscript.Yujie Wang, Jianheng Bao, and Lirong Yin revised the manuscript. Yujie Wang and Jianheng Bao contributed equally to this study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eurox.2024.100303.

References

[1] Ye C, Ruan Y, Zou L, et al. The 2011 survey on hypertensive disorders of pregnancy (HDP) in China: prevalence, risk factors, complications, pregnancy and perinatal outcomes. PLoS One 2014;9(6):e100180.
[2] Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Hypertension 2018 Jun;71(6):e13–115.
[3] Amaral LM, Cunningham Jr MW, Cornelius DC, et al. Preeclampsia: long-term consequences for vascular health. Vasc Health risk Manag 2015;11:403–15.
[4] Mizuno S, Ogishima S, Nishigori H, et al. The Pre-Eclampsia Ontology: A Disease Ontology Representing the Domain Knowledge Specific to Pre-Eclampsia. PloS One 2016;11(10):e0162828.
[5] Agius A, Sultana R, Camenzuli C, et al. An update on the genetics of pre-eclampsia. Minerva Ginecol 2018 Aug;70(4):465–79.
[6] Brown MA, Magee LA, Kenny LC, et al. The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice. Pregnancy Hypertens 2018 Jul;13:291–310.
[7] Harmon AC, Cornelius DC, Amaral LM, et al. The role of inflammation in the pathology of preeclampsia. Clin Sci (Lond, Engl: 1979) 2016 Mar;130(6):409–19.
[8] Passan S, Goyal S, Bhat MA, et al. Association of TNF-α gene alterations (c.-238G>A, c.-308G>A, c.-857C>T, c.-863C>A) with primary glaucoma in north Indian cohort. Gene 2019 Aug 15;709:25–35.
[9] Niktalab R, Piravar Z, Behzadi R. Different Polymorphisms of Vascular Endothelial Growth Factor Gene in Patients with Pre-Eclampsia among The Iranian Women Population. Int J Fertil Steril 2020 Apr;14(1):41–5.
[10] Pazarbaşi A, Kasap M, Güzel AI, et al. Polymorphisms in the tumor necrosis factor-alpha gene in Turkish women with pre-eclampsia and eclampsia. Acta Med Okayama 2007 Jun;61(3):153–60.
[11] Shaw J, Tang Z, Schneider H, et al. Inflammatory processes are specifically enhanced in endothelial cells by placental-derived TNF-α: Implications in preeclampsia (PE). Placenta 2016 Jul;43:1–8.

[12] Canto-Cetina T, Canizales-Quinteros S, de la Chesnaye E, et al. Analysis of C-850T and G-308A polymorphisms of the tumor necrosis factor-alpha gene in Maya-Mestizo women with preeclampsia. Hypertens Pregnancy 2007;26(3):283–91.
[13] Puppala M, Kalpana Veerathu L, Argi A, et al. AB032. Cytokines gene polymorphism in pregnant women at a risk of preeclampsia. Ann Transl Med 2017; 30.
[14] Mohammadpour-Gharehbagh A, Jahantigh D, Eskandari M, et al. The role of TNF-α and TLR4 polymorphisms in the placenta of pregnant women complicated by preeclampsia and in silico analysis. Int J Biol Macromol 2019 Aug 1;134:1205–15.
[15] Zubor P, Dokus K, Zigo I, et al. TNF α G308A gene polymorphism has an impact on renal function, microvascular permeability, organ involvement and severity of preeclampsia. Gynecol Obstet Investig 2014;78(3):150–61.
[16] Saarela T, Hiltunen M, Helisalmi S, et al. Tumour necrosis factor-alpha gene haplotype is associated with pre-eclampsia. Mol Hum Reprod 2005 Jun;11(6): 437–40.
[17] Denney JM, Bird C, Gendron-Fitzpatrick A, et al. Renin-angiotensin system transgenic mouse model recapitulates pathophysiology similar to human preeclampsia with renal injury that may be mediated through VEGF. Am J Physiol Ren Physiol 2017 Mar 1;312(3). F445-f455.
[18] Almasry SM, Elfayomy AK, Hashem HE. Ultrastructure and histomorphometric analysis of human umbilical cord vessels in preeclampsia: a potential role of VEGF, VEGFR-1 and VEGFR-2. Rom J Morphol Embryol = Rev Roum De Morphol Et Embryol 2016;57(2 Suppl):681–9.
[19] Andraweera PH, Dekker GA, Dissanayake VH, et al. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri-Lanka. J Matern-Fetal Neonatal Med: J Eur Assoc Perinat Med, Fed Asia Ocean Perinat Soc, Int Soc Perinat Obstet 2013 Mar;26(5):532–6.
[20] Chedraui P, Solis EJ, Bocci G, et al. Feto-placental nitric oxide, asymmetric dimethylarginine and vascular endothelial growth factor (VEGF) levels and VEGF gene polymorphisms in severe preeclampsia. J Matern-Fetal Neonatal Med: J Eur Assoc Perinat Med, Fed Asia Ocean Perinat Soc, Int Soc Perinat Obstet 2013 Feb;26 (3):226–32.
[21] Rak J. VEGF-D(ilated) lymphatics as gateways to metastasis. Cancer Cell 2012 Feb 14;21(2):139–40.
[22] Amosco MD, Tavera GR, Villar VAM, et al. Non-additive effects of ACVR2A in preeclampsia in a Philippine population. BMC Pregnancy Childbirth 2019 Jan 8;19 (1):11.
[23] Amin-Beidokhti M, Gholami M, Abedin-Do A, et al. An intron variant in the FLT1 gene increases the risk of preeclampsia in Iranian women. Clin Exp Hypertens 2019;41(8):697–701.
[24] Hamid HM, Abdalla SE, Sidig M, et al. Association of VEGFA and IL1β gene polymorphisms with preeclampsia in Sudanese women. Mol Genet Genom Med 2020 Mar;8(3):e1119.
[25] Amosco MD, Villar VA, Naniong JM, et al. VEGF-A and VEGFR1 SNPs associate with preeclampsia in a Philippine population. Clin Exp Hypertens 2016;38(7): 578–85.
[26] Srinivas SK, Morrison AC, Andrela CM, et al. Allelic variations in angiogenic pathway genes are associated with preeclampsia. Am J Obstet Gynecol 2010 May; 202(5). 445.e1-11.